A NONHEREDITARY, HOST-INDUCED VARIATION OF BACTERIAL VIRUSES[1]

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One of virology's most generally valid rules is that the properties of virus particles are unaffected by the host in which they grow. Host adaptation and tissue adaptation, the apparent exceptions, are explained today by selective reproduction of virus mutants in new hosts or in new tissues. In analyzing the relation between certain phages and certain mutants of their bacterial hosts, we have encountered a novel situation: the genotype of the host in which a virus reproduces affects the phenotype of the new virus. The phenotypic change suppresses the ability of the virus to reproduce in certain hosts but not in others. It is a transient change, in the sense that one cycle of growth in a suitable host returns the virus to its original form. Both the growth ability of the modified virus and, in some cases, its production from normal virus are controlled in part by the physiological state of the host cell. The present paper describes these findings and discusses their general implications. Other instances of host-controlled phenotypic changes in bacteriophages have recently been discovered (Bertani and Weigle, 1952).

MATERIALS AND METHODS

The phages employed belong to the T1-T7 system active on *Escherichia coli*, strain B, and on *Shigella dysenteriae*, strain Sh. Standard methods and media were employed (Adams, 1950). Microscopic observations of phage-infected bacteria were done at 30 C or 37 C on nutrient agar plates observed under a dry objective of N.A. 0.66.

In the following sections, the expression "young cells" is used to designate cells in the exponential growth phase in aerated nutrient broth; these generally were used when the viable count was about 10^8 per ml. "Old cells" refers to stationary phase cells in cultures continuously aerated for at least 18 hours (viable count about 4×10^9 per ml).

RESULTS

Bacterial mutants. About 100 independently arisen mutant strains B/4 were isolated at various times from as many separate cultures of B grown from small inocula and plated with an excess of phage T4. After several restreakings, none

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of them was lysogenic for T4. As previously reported (Luria, 1946), these B/4 mutants,[3] when tested as hosts for T2 and T6, fall into three true breeding groups. Some strains (10 out of 94) are as sensitive to T2 and T6 as strain B. Others (59 out of 94) give a somewhat low plate count with T2 and T6 (10 to 50 per cent of the count on B).

The third group (25 out of 94) is the one whose relation with T2 and T6 is described in this paper. The strains of this group will be divided into two subgroups, strain $B/4_0$ and strain $B/4_{00}$; both groups give rise on nutrient agar to characteristic colonies, distinctly rougher than those of *E. coli*, strain B, and its other strain B/4 mutants. They are resistant to T3 and T7 and fully sensitive to T1 and T5. A rare, nonaerogenic, small colony mutant type (strain B/4, 3, 7, 1, tryptophan dependent) behaves like strain $B/4_0$ with regard to T2 and T6. Some $B/4_0$ and $B/4_{00}$ strains, after many subcultures, revert gradually either to the B/4 or to the B phenotype. This reversion has not yet been investigated.

The behavior of strain $B/4_0$ will be described first; then the distinguishing properties of strain $B/4_{00}$ will be listed.

The relation of strain $B/4_0$ with T2 and T6. Phages T2 and T6 give no plaques when plated in moderate amounts with strain $B/4_0$. Plates seeded with at least 10^7 particles of T2 or T6 and up to 10^8 cells of strain $B/4_0$ give a partially lysed layer of growth or an almost complete lysis (with occasional resistant colonies of strain $B/4_0/2$ or strain $B/4_0/6$). These observations suggested lysis of the initially plated bacteria without phage production. Tests in liquid medium revealed the following facts:

1. Strain $B/4_0$ cells adsorb T2 and T6 about as well as strain B cells. The adsorption rate constants per cell are 1 to 2×10^{-9} min^{-1} for young cells, 1 to 5×10^{-10} min^{-1} for old cells.

2. Lysis, as shown by microscopic observation of young cells of strain $B/4_0$ multiple-infected with T2 or T6, occurs at similar times as with strain B and is equally complete. In one experiment (no. 8-4), for example, 72 out of 80 bacteria lysed in 95 minutes. This observation explains the suppression of bacterial growth on strain $B/4_0$ plates with an excess of phage T2 or T6.

3. Phage liberation by strain $B/4_0$ remained uncertain as long as plaques were sought using strain B or its mutants as indicators; the situation was clarified by the discovery that strain $B/4_0$ liberates phage that forms plaques when plated with *S. dysenteriae*, strain Sh, while behaving abnormally toward *E. coli*, strain B. Normal T2 and T6 give equal counts on strain B and on strain Sh.

Results from one step experiments of T2 and T6 on strain $B/4_0$ with platings on strain Sh and on strain B (young cells) are given in table 1. In these experiments the unadsorbed phage was removed by addition of antiphage serum before diluting the adsorption mixture; the only active phage was what came out of the infected cells of strain $B/4_0$. The results show that most and possibly all the young cells of strain $B/4_0$ liberate phage that forms plaques on plates seeded with strain Sh but not on those with young cells of strain B.

[3] T4-resistant mutants are generally B/4, 3, 7, occasionally B/4, 3 (Demerec and Fano, 1945).

TABLE 1

*The production of T*2 and T*6 in bacteria B/4$_s$ and B/4$_{ss}$*

One step growth experiments of T2 or T6 on B/4$_s$ and B/4$_{ss}$. Young or old cells were mixed with phage. After 6 minutes, the mixtures were diluted into antiphage serum to stop adsorption. Four minutes later, when over 99 per cent of the phage that had remained free was neutralized, further dilutions were made and samples were plated with young cells of strain B and with strain Sh. All counts are given in plaques per ml, referred to the adsorption mixtures.

	EXPERIMENT NO.					
	139	139	135	135	138	139
Bacterial strain	B/4$_s$, strain 21	B/4$_s$, strain 21	B/4$_{ss}$, strain 4	B/4$_{ss}$, strain 4	B/4$_s$, strain 21	B/4$_{ss}$, strain 4
Age of bacteria	Young	Old	Young	Old	Young	Young
Phage	T2	T2	T2	T2	T6	T6
Phage input	1×10^7	1×10^7	5×10^7	5×10^7	1.6×10^7	3.0×10^7
Number of infected bacteria that produce plaques on Sh	9.0×10^6	5.7×10^6	2.6×10^7	2.7×10^7	1.1×10^7	2.4×10^7
Number of infected bacteria that produce plaques on young cells of B	6×10^3	1×10^3	2.5×10^5	2.3×10^7	5×10^3	2.5×10^5
Titer after lysis, on strain Sh	7.0×10^7	5.0×10^6†	4.0×10^8	5.4×10^7	1.5×10^8	2.7×10^8
Titer after lysis, on young cells of B	2.5×10^3	2×10^3	1.7×10^5	5.1×10^7	5×10^3	1.3×10^5
Average yield per cell, T*	8	1†	15	—	14	11
Average yield per cell, T2	—	—	—	2	—	—

† Phage liberation was probably not completed at the time of plating.

When old strain B/4_0 cells are infected with T2 or T6, only about half the cells produce some phage which is active on strain Sh but not on young cells of strain B. The average yield per young cell of strain B/4_0, measured on strain Sh, is 8 to 30 for T2 and T6; for old cells the yield is 1 to 4. Temperature and nature of the medium in which strain B/4_0 cells have been grown do not affect the nature of the phage they produce.

The type of experiment shown in table 1 defines the modified form T* of the phages T2 and T6 (T*2 and T*6, respectively) as one produced in strain B/4_0 and forming plaques on strain Sh but not on young cells of strain B. The properties of T* will be described later.

The plaque counts obtained in platings of infected strain B/4_0 bacteria on young cells of B (table 1), although low, are significantly higher than can be accounted for by any serum-surviving free phage from the input. This might mean that a few strain B/4_0 cells (less than 1 in 1,000) liberate some normal phage particle. More probably it means that one out of many thousands T* particles plated has an opportunity of producing a plaque on the B plates, possibly because on the plate it only becomes adsorbed late when some of the strain B cells have aged and become competent to support growth of T* (see below).

The relation of strains B/4_{00} with T2 and T6. Young cells of B/4_{00} strains infected with T2 and T6 behave somewhat similarly to young cells of strain B/4_0 (table 1). About 97 to 99 per cent of the cells liberate only T* phage, active on strain Sh but not on strain B. About 1 to 3 per cent of the infected bacteria form plaques on young cells of B; the yield of phage that can form plaques on young cells of B is of the order of one or two per cell.

Old cells of strain B/4_{00} infected with T2 or T6 liberate normal T2 or T6 in small amounts, similar to those produced by old cells of B (table 1). In most experiments there is evidence for the production of some T* phage along with the T phage, as evidenced by higher counts on strain Sh than on young strain B cells (tables 1 and 4). The amounts of T* produced are variable, from less than 20 per cent of the yield to about one-third of it.

Thus, old cultures of strain B/4_{00} appear to contain some organisms resembling the young cells in their ability to produce T* from T phage; young cultures of B/4_{00}, on the other hand, contain a few cells that can liberate some T phage as such. The liberation of T* or T form of phage from B/4_{00} cells in the phase of transition from old age to log phase and vice versa has not yet been analyzed.

*Properties of T*2 and T*6.* Qualitatively, T*2 and T*6 behave in similar ways. Since most experiments were done with T*2—the critical ones being confirmed with T*6—we shall use the T2-T*2 system as a model.

Phage T*2 is neutralized by anti-T2 serum at the same rate as T2. It is as stable *in vitro* as T2. It is not converted to T2 *in vitro* by treatment with distilled water, which raised the titer of fresh T2 lysates by removing a phage inhibitor of bacterial origin (Bertani and Sagik, to be published). T*2 lysates can be prepared by infecting strain B/4_0 with T2, removing most unadsorbed T2 by centrifugation, washing the infected cells before lysis, and allowing them to lyse in nutrient broth without added NaCl; readsorption of the liberated phage is

thereby prevented. The best lysates gave counts about 1,000-fold higher on strain Sh than on young strain B plates. The count on Sh cells may be due partly to some residual T2 particles, partly to some T*2 particles that happen to infect bacteria late when the cells have become old on the plate (see item 4, below).

1. T*2 is adsorbed by strain Sh and lyses it, giving an apparently pure yield of normal T2. The adsorption of T*2 by strain Sh is similar to that of T2; the yield of T2 is the same whichever phage form is the infecting one (table 2). The liberation of T2 by bacteria infected with T*2 will be called "activation" of T*2.

2. T*2 is adsorbed by cells of strains B, B/4, B/6 (not by strain B/2). One particle per cell is sufficient to prevent colony formation by strain B cells. T*6 is adsorbed by strain B/2, not strain B/6.

TABLE 2

*One step growth experiments with T*2 and T2 on strain Sh*

Experiment no. 154. Young cells of strain Sh (10^8 cells per ml) were mixed with phage. After adsorption the free phage was eliminated with antiserum and after dilution platings were made at intervals before and after lysis. All counts are given in plaques per ml of the adsorption mixtures.

	PHAGE	
	T*2	T2
Phage input	4×10^7	1×10^8
Plaque count before lysis, on young cells of B	2.2×10^7	5.6×10^7
Plaque count before lysis, on cells of Sh	2.5×10^7	5.9×10^7
Plaque count after lysis, on young cells of B	2.8×10^9	6.0×10^9
Plaque count after lysis, on cells of Sh	2.6×10^9	6.0×10^9
Average yield of T2 per cell	115	105

3. If young cells of strain B that have adsorbed T*2 are plated with an excess of either strain B or strain Sh, about 99.9 per cent of them produce no plaques (no activation nor reproduction of T*2 as such; see table 3). Microscopic observation at 30 C shows that the infected cells do not divide; many of them elongate, reaching lengths of 20 μ or more, then slowly disintegrate.

4. If old cells of strain B are infected with T*2 at 37 C, about 1 per cent of them liberates T2 (equal counts on young strain B and on strain Sh plates; see table 3). The yield appears to consist of T2 phage only, without admixed T*2. The fraction of old strain B cells that activates T*2 is not greatly different, whether the cells have been infected in their old culture medium, or after washing and resuspending in buffer, or after washing in buffer and resuspending in fresh broth containing T*, provided T* adsorption occurred promptly after contact with fresh medium. The remaining cells liberate nothing. The fraction of old cells of strain B that activates T*2, whether in buffer or in the exhausted medium, is

higher when infection takes place at lower temperatures—for example, 3 per cent at 25 C, 1 per cent at 37 C, 0.5 per cent at 43 C (experiment no. 123).

5. If old strain B cells are resuspended in fresh aerated broth and preincubated before adding T*2, the fraction of infected cells that activates T*2 depends on the time and temperature of preincubation. A set of representative results is shown in figure 1, a. Upon preincubation at 13 C little change occurs in 2 hours. At 25 C the fraction of strain B cells that activates T*2 increases at first, then slowly decreases. At 37 C there is no increase and the decrease is more rapid. At 43 C the cells lose their activating ability in a few minutes and afterward behave like young cells of strain B. Preincubation of old cells of strain B in buffer

TABLE 3

*Test for phage liberation by various types of cells infected with T*2*

Experiment no. 148. A lysate of T*2 was mixed at 37 C with cells of various strains, either young or old. After allowing 4.5 minutes for adsorption, the mixtures were treated with antiphage serum, then diluted and plated. The concentration of old cells was 6×10^8 per ml, that of young cells 2×10^8 per ml. The phage lysate had a plaque titer of 1.2×10^9 on strain Sh and 4.3×10^8 on young cells of strain B.

	PLATINGS ON YOUNG CELLS OF B		PLATINGS ON CELLS OF Sh		NATURE OF YIELD
	Count	Per cent of T*2 input	Count	Per cent of T*2 input	
PHAGE INPUT	4.3×10^4	0.036	1.2×10^6	100	
Bacterial strain					
Sh	9.0×10^7	75	7.5×10^7	63	T2
Young B	7.2×10^4	0.06**	7.2×10^4	0.06**	—
Old B	5×10^5	0.4	6×10^5	0.5	T2
Young B/4_0	$<4 \times 10^1$	—	4×10^4	0.03**	—
Old B/4_0	$<5 \times 10^1$	—	5×10^5	0.4	T*2
Young B/4_{00}	1.4×10^3	0.001	1.9×10^5	0.16	T*2
Old B/4_{00}	1.9×10^6	1.6	2.6×10^6	2.2	T2, T*2

** These ratios are not significantly different from the ratio between counts on young cells of B and on cells of Sh in the input phage (0.036).

at various temperatures for up to 90 minutes does not reduce their ability to activate T*2.

6. When young cells of strain B/4_{00} are infected with T*2, over 99 per cent of the cells yield nothing, just as young cells of strain B (see table 3). Specially designed experiments showed that of the few cells that liberate something, almost all yield only T*2, but a few yield some T2 just as if they had been infected with T2 (see table 4).

If old cells of strain B/4_{00} are infected with T*2 at 37 C, about 1 to 3 per cent activate T*2, as old cells of strain B would (table 3). The dependence of the fraction of cells of B/4_{00} that activates T*2 on the time and temperature of preincubation in fresh medium is qualitatively similar to that observed with old strain B (see figure 1, b). The old strain B/4_{00} cells often liberate some phage

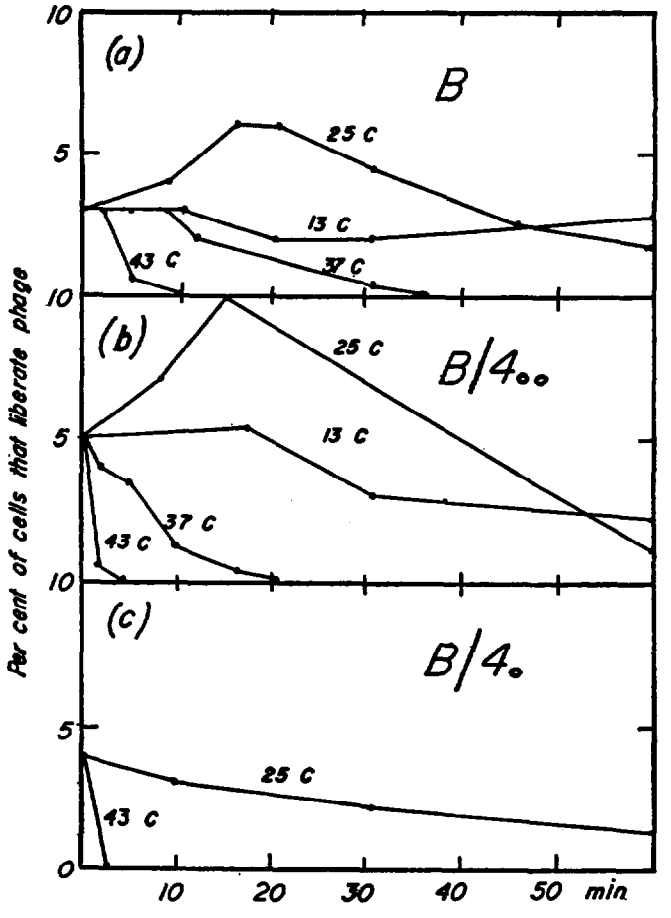

Figure 1. The proportion of old cells that liberates phage after infection at 25 C with T*2, as a function of the time and temperature of incubation in fresh aerated nutrient broth before infection. Strain B (diagram a) liberates T2. Strain B/4∞ (diagram b) liberates T2 with some T*2. Strain B/4₀ (diagram c) liberates T*2.

TABLE 4

*Comparison of the production of T2 and T*2 by B/4∞ bacteria, strain 4, infected with T2 or T*2*

EXPERIMENT NO.	143		145	
AGE OF BACTERIA, STRAIN B/4∞	Old		Young	
PHAGE	T2	T*2	T2	T*2
Phage input	5.3×10^6	9.0×10^7	1.4×10^7	1.2×10^7
(a) Platings before lysis, on young cells of B	3.4×10^6	3.4×10^6	1.0×10^5	1.1×10^5
(b) Platings before lysis, on cells of Sh	4.5×10^6	3.8×10^6	8.0×10^6	9×10^5
Ratio (a)/(b)	0.75	0.89	0.012	0.012
(c) Platings after lysis, on young cells of B	1.8×10^7	1.2×10^7		
(d) Platings after lysis, on cells of Sh	2.7×10^7	1.9×10^7		
Ratio (c)/(d)	0.67	0.63		

T*2 along with the T2, just as they do when infected with T2. The relative amounts of T*2 and T2 liberated by a given culture of strain B/4_{00} are the same whether the cells are infected with T2 or T*2 (see table 4). Altogether, old B/4_{00} cells resemble old B cells with regard to ability to produce T2 from T2 and T2 from T*2. However, they also produce some T*2 from either T2 or T*2.

7. When young cells of strain B/4_0 are infected with T*2, at least 99.9 per cent yield nothing (see table 3). When old cells of strain B/4_0 are infected with T*2 a small fraction of them liberates T*2, the others liberate nothing (table 3). Age and temperature relations in the reproduction of T* by old strain B/4_0 broadly resemble those in the activation of T*2 by old strain B or strain B/4_{00} (figure 1, c).

In summary, T*2, produced in strain B/4_0 or in strain B/4_{00}, is adsorbed by and kills the bacterial strains susceptible to T2. It grows in every cell of strain

T2 + Shiga ⟶ T2	T*2 + Shiga ⟶ T2
B young ⟶ T2	B young → –
B old ⟶ T2	B old ⟶ T2
B/4_0 young ⟶ T*2	B/4_0 young → –
B/4_0 old ⟶ T*2	B/4_0 old → T*2
B/4_{00} young ⟶ T*2	B/4_{00} young → T*2
B/4_{00} old ⟶ T2+T*2	B/4_{00} old → T2+T*2

Figure 2. Results of infection of various bacterial strains with phages T2 and T*2.

Sh and in a fraction of old B cells, giving a yield of normal T2. It grows in a fraction of old cells of strain B/4_0, giving a yield of T*2. It grows in a fraction of old B/4_{00} cells, giving a yield that consists mainly of T2, but contains some T*2. It grows in a very small fraction of young cells of strain B/4_{00}, giving rise to production of T*2. The properties of T*6 are analogous, *mutatis mutandis*, to those of T*2. The r mutants behave like their parent phages. These facts are summarized in the scheme of figure 2. It is apparent that T*2 is distinguished from T2 by the fact that it requires for growth a physiologically "competent" host. Competence may involve ability to produce T2 from T*2 (in old strain B, in old strain B/4_{00}, and in strain Sh) or to produce T*2 from T*2 (in old strain B/4_0).

It is important to note that whenever T*2 initiates phage production in a given type of bacteria, the composition of the yield obtained, if any, is exactly the same as if the infecting phage had been T2. There is no evidence of any influence of the

T or T* quality of the infecting phage on the quality of the progeny phage, which appears to be exclusively host controlled.

*Interactions of T*2 in mixed infection.* 1. *Genetic recombination.* Mixed infection of young cells of strain B with equal amounts of T*2 and T2hr gives a yield containing T2, T2r, T2h, and T2hr (table 5). The recombinant types are more numerous than the parental type resembling the T*2 parent. The number of bacteria that liberates phage with genetic characteristics of the T* parent is significantly higher than the number of bacteria infected with any residual T2 particles in the T*2 lysate, but is less than one-tenth the number of bacteria infected with both T*2 and T2hr. Thus, the T* parent behaves in mixed infection

TABLE 5

*Genetic recombination between T*2 and T2hr*

Experiment no. 109. Equal amounts of T*2 and T2hr sufficient to give multiple infection (3 phages of each type per cell) were mixed separately or together with young cells of strain B in nutrient broth. After 6 minutes for adsorption and 4 minutes of antiserum treatment, the mixtures were diluted and assayed at intervals for plaque counts of T2, T2h, T2r, T2hr, either on young strain B or on a mixture of young cells of B and young cells of B/2.

	CULTURE NO.		
	1	2	3
Phage	T*2	T2hr	T*2 + T2hr
Platings before lysis; plaque count on strain B	*1.5* r†	709 r	521 r 40 mottled (mixed r and r†)
Platings after lysis, dilution 1:40; plaque count on strains B + B/2	—	546 hr	495 hr 22 h†r 16 hr† 2 h†r†

The count in italics was obtained by plating a more concentrated sample and correcting for the difference in concentration.

as a minority parent, as though only a fraction of the T* particles could contribute to recombination in mixed infected bacteria.

2. *Mutual exclusion.* Mixed infection of young strain B with T*2 and T6, with T*2 preceding T6 by 5 minutes, reduces the number of cells that liberates T6, indicating that T* can to some extent exclude a related heterologous phage. The reduction is less than expected if every adsorbed particle of T*2 could exclude T6.

The partial sensitivity of strain B/4$_{00}$ to T4. Most B/4 strains, including those of the strain B/4$_0$ group, are fully resistant to T4, which they fail to adsorb, at least in the irreversible step (Garen and Puck, 1951). Strains B/4$_{00}$, however, show an age dependence in their resistance to T4 (two strains tested). Most old cells, plated on nutrient agar coated with an excess of T4, fail to form colonies; about 2 to 20 per cent as many colonies are formed on phaged plates as on non-phaged ones. The old cells adsorb T4 in liquid, but liberate none. Microscopic

observation shows that most cells fail to increase in size and to divide; many of them slowly fade away. If old strain B/4_{00} cells are incubated in fresh aerated broth at 37 C and samples are plated at intervals on plates with and without T4 (see figure 3), the proportion of colonies formed on T4 increases slowly. A comparison of figures 3 and 1,b shows that the process of "rejuvenation" that suppresses the susceptibility of strain B/4_{00} to T4 is much slower than the one that suppresses the ability to activate T*2. Preliminary experiments indicate, on the other hand, that the ability of old strain B/4_{00} to produce T2 from T2 is lost during rejuvenation at about the same rate as the susceptibility to killing by T4.

The few colonies that appear after plating old strain B/4_{00} cells on T4 probably do not stem from genetically different cells; in fact, subcultures from these colonies give the same ratio of colony counts on plates with and without T4 as the

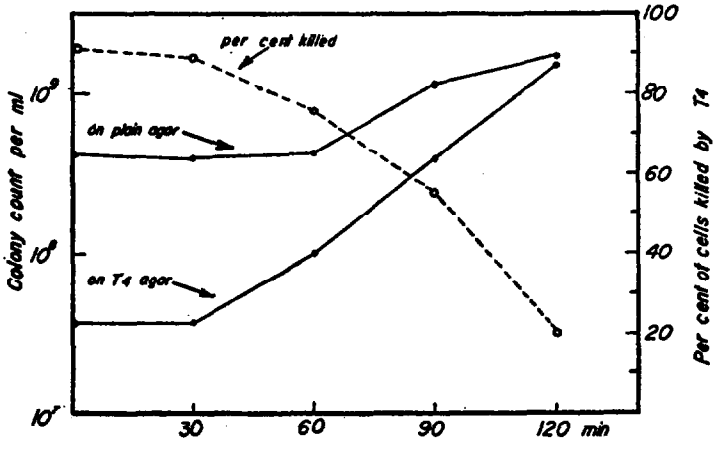

Figure 3. Changes in the proportion of strain B/4_{00} cells killed by T4 as a function of the time of incubation in fresh aerated nutrient broth at 37 C before plating for colony count. Platings with T4 were done by mixing the bacterial samples with T4 (2×10^{10} particles per ml) and plating 0.1 ml aliquots.

original platings. The colonies formed on T4 plates may represent cells that, by chance, fail to meet T4 until they are rejuvenated.

*Attempts to activate free T**. Although the findings listed before indicate that activation of T* results from adsorption and growth in a competent bacterium, a variety of tests was done in an effort to eliminate the possibility that activation of T* in a mixture with bacteria be due, not to adsorption, but to the action of some soluble product of bacterial cells. All tests were negative. Filtrates and supernatants of strain Sh or of old strain B cultures were ineffective under all conditions and temperatures tested. Suspensions of washed cells of strain Sh or old strain cultures of B, resuspended in buffer, were about as effective in activating T* as the whole culture from which they came. Activation only occurred if T* was mixed with cells under conditions where adsorption was possible. Also, the fact that activation fails with resistant bacteria (strain B/2 for T*2; strain B/6 for T*6) would require unwarranted assumptions to be reconciled with the idea of extracellular activation.

DISCUSSION

Production of the T form of phage.* The T* form of phages T2 and T6 is produced only in some bacterial mutants (strains B/4_0 and B/4_{00}) resistant to T4, T3, and T7. The relation between the resistance to these phages and the modifying influence on T2 and T6 is unknown, as is also the relation between acquisition of resistance to certain phages and changes in the nutritional requirements (Anderson, 1946; Wollman, 1947) or in the growth rate of bacteria (Luria, 1946). A physiological connection between resistance to T4 and modifying action on T2 and T6 is suggested by the fact that in the B/4_{00} strains the old cells are both capable of reproducing some T2 without modifying it and susceptible to killing by T4. These two properties of old cells disappear at similar rates during their rejuvenation in fresh medium.

The remarkable fact is the role played by the host's heredity and by its physiological state in determining the properties of the virus it liberates. This evidences a hitherto unsuspected plasticity of virus properties at the phenotypic level; it suggests that virus physiology, as expressed in the various phases of interaction with the host cell, may be controlled in part by nongenetic, "cytoplasmic" components of the virus particles. The existence of physiologically active, nongenetic components in some bacteriophages has become increasingly probable because of a number of recent findings (Benzer, 1952; Dulbecco, 1952; Luria, 1952; Hershey and Chase, 1952).

Control by the genetic and developmental make-up of the host cells over the future reproductive ability of virus particles liberated from them could play a tremendous role in determining the course of virus infections, both at the epidemiological and the pathological level. We may mention that some of the findings on the system T2-T*2 provide an analogy for the cases of "masking" in some virus diseases of animals, for example, in rabbit papilloma where infectious virus with capacity for indefinite transmission cannot be isolated from the tumors of the domestic rabbit, but only from the cottontail rabbit (Shope, 1950). The T*2 form produced in strains B/4_0 or B/4_{00} cannot be detected with actively metabolizing cells of strains B or B/4; only the availability of strain Sh permitted its identification.

The role of the physiological state of strain B/4_{00} in determining the alternative T*2 or T2 phenotypes suggests by analogy the interesting possibility that a developmentally specialized animal tissue may so modify a virus particle as to render it incapable of reproduction in that tissue itself or in some other tissue, thus providing for an active rather than a passive determination of virus tropisms by the host. Speculative as these analogies are, we may gain from an awareness of their implications. The possible occurrence of host-induced restrictions in the host range of viruses should be kept in mind, for example, in considering the role of viruses in the etiology of "nonvirus" tumors.

Growth phase control of the virus-producing ability of bacteria. The T* form of phage, when infecting *E. coli* host cells, fails to multiply in young, actively metabolizing cells but multiplies in an appreciable fraction of old, starved bacteria. Considering the scheme of figure 2 we see that in "competent" cells, where it can reproduce, T*2 gives rise to a progeny identical to that which T2 would

give in the same host. The quality, T or T*, of the phage that is produced appears to depend only on the host, not on the quality of the infecting phage. These phage changes clearly are not heritable since there is no cell type in which the composition of the yield is determined by the infecting phage.

Guélin (1948) described the lysis of several *E. coli* strains by phage C 16 without production of phage; C 16 reproduced normally in several dysentery organisms. It is possible that C 16, a phage related to T2 and T6 (Adams, 1952), gave rise in *E. coli* to a progeny analogous to T*, but not revealed by any of the host organisms tested in Guélin's work.

In view of recent evidence for a stagewise process of phage maturation (Luria, 1952), it is tempting to visualize T* as a somewhat imperfect form of the phage, resulting possibly from an inadequate supply of some essential component in the strain B/4_0 host. The defect would manifest itself as an inability to perform successfully some step needed for production or liberation of active phage in young cells of *E. coli*, strain B, and of its mutants. In the competent cells of strain Sh and of old strain B cultures, either the defective phage activity may not be needed or its performance may only be required at an efficiency level inadequate for success in the young cells of strain B. Although we do not know at what state the process of phage production is stopped in young cells of *E. coli* infected with T*, the low recombination and exclusion efficiencies of T*2 suggest that the block does not concern simply the process of lysis and phage release.

The data presented in this paper on the influence of the physiological state of strain B cells on the growth of T* do not permit a choice among a number of possible hypotheses as to what phases of host metabolism are involved in the transition of strain B cells from competence to incompetence and vice versa. The proportion of competent cells is affected by time and temperature of preincubation of old cells in fresh nutrient medium, but not in buffer. Evidently an active metabolism is required for the transition from competence to incompetence as for the concomitant transition from resting phase to active growth phase. The complex effects of the time and temperature of preincubation on the proportion of competent cells suggest an interplay of various reactions with different temperature dependences.

SUMMARY

Several B/4 mutants of *Escherichia coli*, strain B, when infected with phages T2 or T6, liberate these phages in a form designated as T*, which does not multiply in young cells of strain B or of its mutants. T* can multiply in a small proportion of old, starved cells of strain B, giving rise to a yield of the corresponding normal T phage. T* can be transmitted serially as such in a small fraction of the old cells of some B/4 mutants. In *Shigella dysenteriae*, strain Sh, the T* particles behave as normal T particles; infection of a strain Sh cell with a T* particles gives a full yield of normal T phage. These findings reveal an as yet unsuspected susceptibility of viruses to transitory physiological changes induced by the host in which they have grown.

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